Chiton integument: Metamorphic changes in *Mopalia muscosa* (Mollusca, Polyplacophora)

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Summary:
The larval integument and juvenile girdle integument of *Mopalia muscosa* (Mollusca: Polyplacophora) were studied by light microscopy. Within 24 h of settlement, eight distinctive changes occur that characterize metamorphosis: loss of the functional prototroch and apical tuft, secretion of a cuticle over the mantle field followed by the secretion of calcareous shell plates and the extrusion of spicules into the cuticle, a 20% decrease in length, secretion of chitinous hairs and the incorporation of the lateral ciliated bands into the pallial grooves. Similar changes which were often not recognized as metamorphic have been reported for other species. Evidence for metamorphosis being a common developmental feature of chitons is presented.

Article:
A. Introduction
Planktonic larvae of benthic marine invertebrates are anatomically distinct from the juveniles and adults. In most molluscs, drastic and irreversible metamorphic changes transform a larva into a juvenile during a brief part of the life cycle (Hadfield 1978; Bonar 1978), and typically occur after a larva has settled onto the substratum (Thorson 1961,1966).

Thorson (1946) and Christiansen (1954) suggested that chiton larvae do not metamorphose, but instead slowly attain a juvenile shape. Pearse (1979) also stated that metamorphosis in chitons "is not well delineated and mainly involves growth." However, other investigators have found distinct differences between larval and juvenile chitons (Kowalevsky 1883; Hammarsten and Runnstrom 1926; Barnes 1972; Watanabe and Cox 1975), although these differences were not always identified as being part of metamorphosis.

I raised *Mopalia muscosa* in the laboratory from fertilization to over one year of age. My examination of larval and juvenile integuments revealed morphological transformations occurring within 24 h after settlement. In this paper I describe some striking differences between larvae and juveniles that can be considered part of the metamorphic events occurring in this species. I also review evidence from the literature supporting the occurrence of metamorphosis in other chitons.

B. Materials and methods
Adult *Mopalia muscosa* (Gould, 1846) were collected from Cattle Point, San Juan Island, WA during 1977-1979. Animals maintained themselves by grazing on the algae that grew on the walls of the sea tables at the Friday Harbor Laboratories. Eggs were collected from naturally spawning individuals. Viable spermatozoa were collected from either freely spawning individuals or from the dissected testes of adult males. Fertilized eggs were cultured in pyrex containers in sea tables at temperatures between 9°-13° C. A mono-layer of eggs (in a dish) was covered by no more than 3-4 cm of sea water, that was changed daily. Special care was taken, after hatching, to remove the discarded chorions from the culture to prevent bacterial overgrowth. Cultures were washed by allowing eggs to settle and decanting the water or by gently pouring the egg suspension or larvae into a 220 gm mesh sieve.

When larvae became dorso ventrally flattened and spent time motionless on the bottom, they were considered competent to settle. Small rocks covered with red or brown algal films were than added to the culture dishes. Stones with a film of the red alga *Hildenbrandia* sp. best promoted settlement and juvenile growth (Leise, unpublished). Settling individuals were not disturbed for 48 h or until all animals had settled out of the water. Animals were raised from fertilization to one year of age on these small stones in finger-bowls.

Animals were fixed and embedded for light microscopy following the protocol given in Leise and Cloney (1982). Juveniles were decalcified after primary fixation by the addition of an equal volume of 10% disodium EDTA to the primary fixative solution. This 1 :1 solution was replaced every 12 h until decalcification was complete. After decalcification, specimens were postfixed and embedded as de-scribed in Leise and Cloney (1982). One micrometer sections were stained with Richardson's solution (Richardson et al. 1960).

Table 1. Major developmental events in populations of *Mopalia muscosa* reared at Pacific Grove, California (Watanabe and Cox 1975) and Friday Harbor, Washington. Times listed represent entire cultured populations and do not indicate individual variation. Fertilization is time 0

Event	Time at Pacific Grove *(13.5°–15.8° C)*	Time at Friday Harbor *(9–13° C)*
Hatching	20.0 h	24–36 h
Foot development		
initial bulge	6.5 days	5 days
ciliated anterior flap	7.0 days	?
well-developed foot	10.0 days	10 days
Spicule formation		
pretrochal	5.5 days	6 days
encircling mantle	6.5 days	6 days
Shell plates deposited	13.5 days	10–11 days
Settlement	9–10 days	9–10 days
Prototroch disappears	12.5 days	12 days

C. Results

Table 1 compares major developmental events of *Mopalia muscosa* larvae raised in California (Watanabe and Cox 1975) and Washington. Minor differences in the schedule of these events between the two populations were probably caused by differences in temperature or metabolic rates.

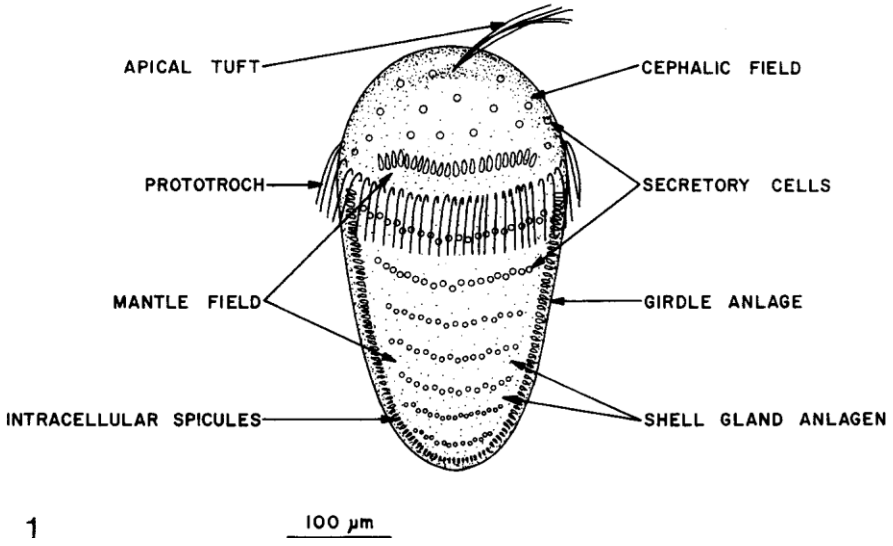

Fig. 1. Diagram of a dorsal view of a trochophore larva that is competent to settle. Within the mantle field, the shel gland anlagen alternate with rows of large secretory cells

A five day-old trochophore is barrel-shaped and about 350 µm long (Fig. 1). Its epidermis is a simple columnar epithelium (Fig. 2a), without the papillae of the adult epidermis (Leise and Cloney 1982). In addition to the prototroch and apical tuft, four regions of the epidermis are ciliated : the cephalic field around the apical tuft,

the foot and a pair of lateral bands just ventral to the mantle field (Figs. 1, 2). Each lateral band runs the length of the posttrochal region along one side of the larva; they meet caudally. The ciliated cephalic field covers the entire pretrochal region except for a dorsal area just anterior to the prototroch. The dorsal surface of the posttrochal region also lacks cilia. These two dorsal, unciliated regions comprise the mantle field (Figs. 1, 2). The cells of the girdle anlage, at the circumference of the mantle field, are about 30 µm high and 4 µm wide (Fig. 2). No spicules are present at this stage. All of the epidermal cells, including the ciliated ones, have an apical fringe of uniform microvilli as a brush border. The epidermal cells retain this brush border until metamorphosis. A cuticle as is seen in the juvenile is not present. Large goblet cells containing mucus are scattered throughout the epidermis, but not among the prototrochal cells. The epidermis of the ventral foot is a ciliated bulge (Fig. 2).

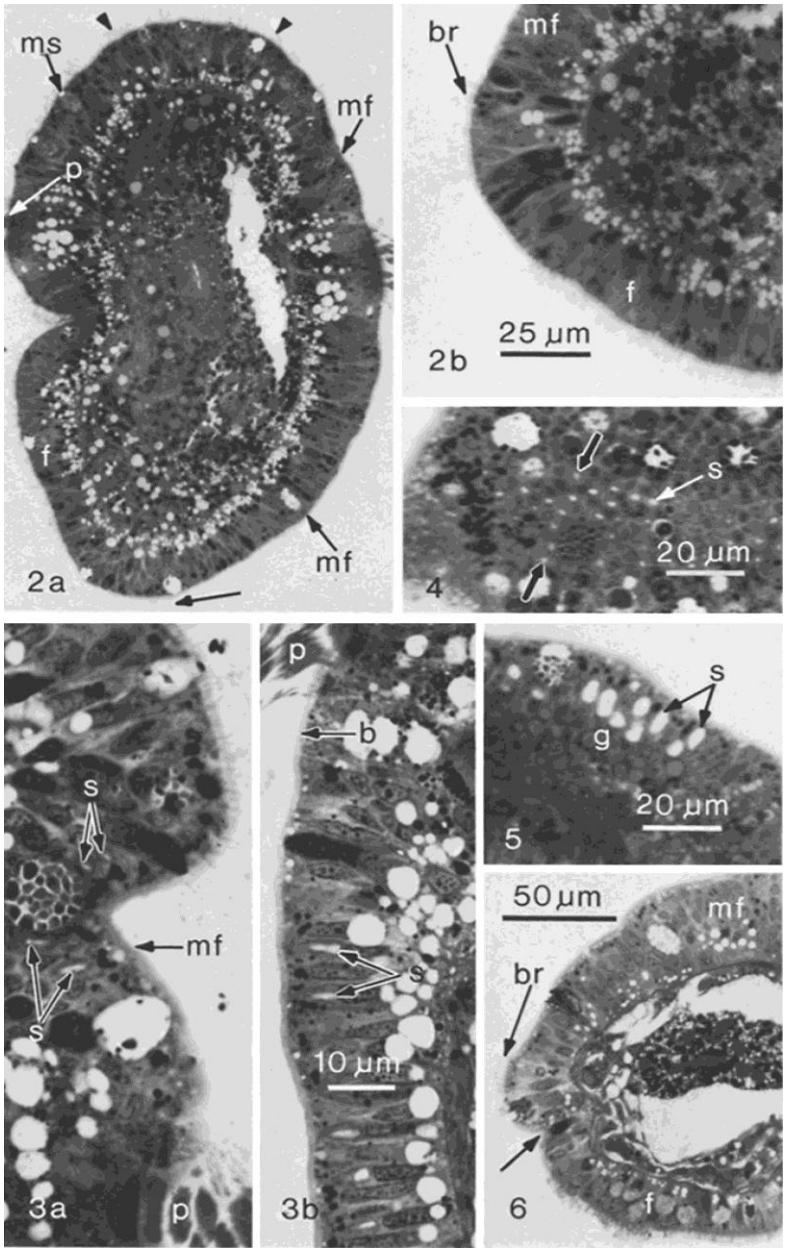

Fig. 2. ***a Longitudinal section through a five day-old trochophore larva. The pretrochal region is ciliated (arrowheads) except for the mantle field (inn. Larvae lack spicules at this stage. The posttrochal mantle field (inf) is also unciliated. Two ciliated bands of cells meet caudally (arrow). × 300. b Transverse section through part of a five day-old trochophore. The ciliated ventral foot (f) is separated by a few unciliated cells from one of the lateral bands (br). The dorsal mantle field (m.f) contains the shell gland anlagen centrally and the girdle anlage peripherally. The ciliated bands (br) border the edge of the girdle anlage. × 550***

Fig. 3. ***a Decalcified longitudinal section through the pretrochal region of a six day-old trochophore. The cells of the mantle field are unciliated (mf). The spiniferous cells are part of the presumptive girdle and***

occur in a band, five or six cells across. Four intracellular spicules (s) are visible in this section. × 710 b Longitudinal section through the presumptive girdle of the posttrochal region of a six day-old larva. Five spiniferous cells contain cuneiform, supranuclear spicules (s). × 950

Fig. 4. *Grazing section through the posttrochal region of the presumptive girdle in a seven day-old larva. The spicules (s) are still intracellular. The band of spiniferous cells remains six cells wide (between arrows). × 570*

Fig. 5. *Longitudinal section through the girdle anlage (g) of a 10 day-old unsettled larva. Although the spicules (s) have greatly increased in size (c.f., Fig. 3 b) they remain intracellular. × 560*

Fig. 6. *Transverse section through part of a nine day-old larva. Each lateral ciliated band (br) is now on a slight protruberance. The foot (1) is cut off from these protruberances by shallow grooves (arrow). × 290*

Some of the cells around the edge of the mantle field of six day-old larvae bear calcareous primary spicules (Fig. 3), a characteristic that distinguishes the presumptive girdle from the anlagen of the shell glands (Fig. 1). These primary spicules are intracellular, about 6 μm × 1 gm, conical, and supranuclear (Fig. 3). A spiniferous cell produces only one spicule. Five to six rows of spiniferous cells ring the entire mantle field (Fig. 4). The spicules remain intracellular until the larva settles, during days nine or 10. The spicules increase in size until they are about 9.0 μm long and 3.0 μm wide just before settlement (Fig. 5). Although the cells of the girdle are still columnar at six days, they become shorter (20 μm) until papillae differentiate.

After nine or ten days, the free-swimming trochophore is ready to settle and metamorphose. At this stage the larva is about 400 gm long and dorsoventrally flattened. The shallow grooves around the foot are more distinct and each lateral ciliated band lies on an epidermal bulge just ventral to the girdle anlage (Fig. 6).

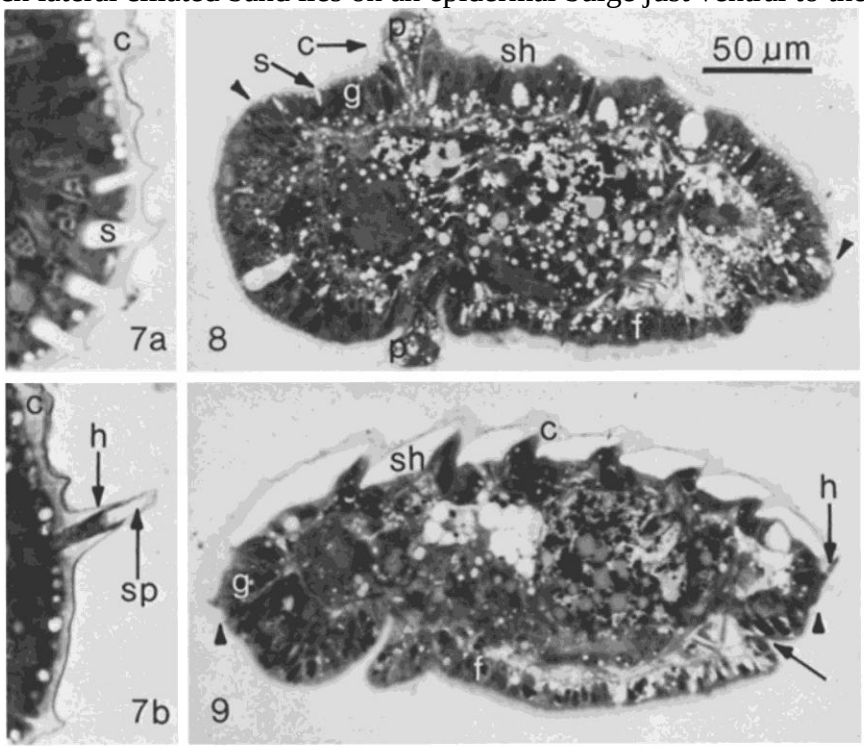

Fig. 7. a Transverse section through the girdle of a decalcified 10 day-old metamorphosed juvenile. A cuticle (*c*) now covers the girdle. It is thinnest ventrally. The ventral surface is directed down in this micrograph. The spicules (*s*) have been partially extruded and their distal ends lie within the cuticle. × 1170. **b** Transverse section through the anterior girdle of the same individual. Small hairs (*h*), each capped by a spikelet (*sp*), have been secreted. The hairs also lie within the cuticle. × 1070

Fig. 8. Sagittal section through a decalcified 11 day-old juvenile. This animal had not completely lost the prototroch (*p*). The cuticle (*c*) covers the shell plates (*sh*) and the epidermis of the girdle (*g*). The posterior girdle is lateral, but the anterior girdle has yet to move to its lateral position. The termination of the cuticle (*arrowheads*) marks the edge of the girdle. × 300

Fig. 9. Sagittal section through a 13 day-old juvenile. This animal has completely lost the prototroch. The posterior and anterior edges of the girdle are now lateral and end where the cuticle stops (*arrowheads*). The foot (*f*) is demarcated from the girdle by a groove (*arrow*). × 310

Within 24 h after settlement, the larva shortens to about 310 gm and transforms into a juvenile. The animal loses the prototroch and apical tuft, secretes a cuticle (Fig. 7), the shell plates (Fig. 8) and hairs (Fig. 7 b), and extrudes the primary spicules (Fig. 7a). Shell is deposited below the cuticle in the central part of the mantle field (Fig. 8), and peripherally the tips of the girdle spicules protrude into it (Fig. 8). Within two days, the foot is demarcated from the girdle by the pallial grooves (Fig. 9). The pallial grooves are situated where the lateral ciliated bands once were. The loss of the prototroch may take 3 or 4 days and can continue as the shell plates coalesce. The change in shape and size of a larva is dramatic (Fig. 10). Old trochophores are pyriform, with a blunt anterior end. As they metamorphose, they become oval. They continue to shorten for 3 or 4 days, as they develop, but start to elongate (Fig. 10) when they begin to feed, after their stored yolk is exhausted.

The cuticular surface of young juveniles is scalloped. Some crenulations correspond to spicule tips or hairs (Fig. 11). At first, only the tips of the spicules lie within the cuticle, but by two days after settlement the spicules are completely extruded and enveloped by the cuticle. The cuticle is thick dorsally and thin ventrally. Primary spicules have thin cups and do not possess the dense chitinous cup, shaft, or central pigmented granules of adult spicules (Leise and Cloney 1982).

Secretion of small hairs also begins at metamorphosis. A fringe of hairs is produced around the perimeter of the girdle in 10 day-old settled animals (Fig. 12). Laterally, one hair grows at each suture, i.e., at each point of junction between two adjacent shell plates (Fig. 13). These hairs are about 2 pm in diameter and 15 pm long, 24 h after settlement. Each hair is covered by a thin cuticular extension.

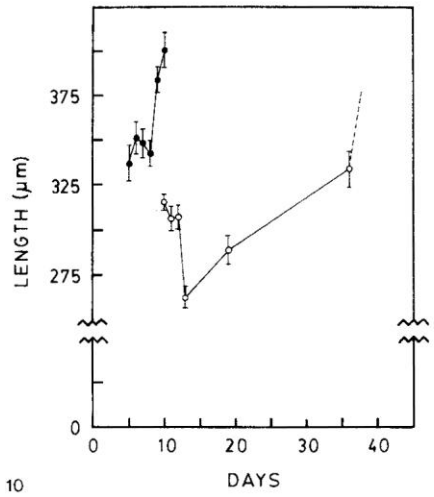

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Fig. 10. Change in length of trochophore larvae (*closed circles*) and metamorphosed juveniles (*open circles*) with time. Each datum represents the mean of 15 individuals, except for the last larval datum, which represents the mean of eight individuals. All measurements were made on material fixed for electron microscopy. The difference between the mean lengths of settled and unsettled 10 day-old animals was found to be significantly different with a two tailed t-test, $p \ll 0.001$. Vertical bars are standard error

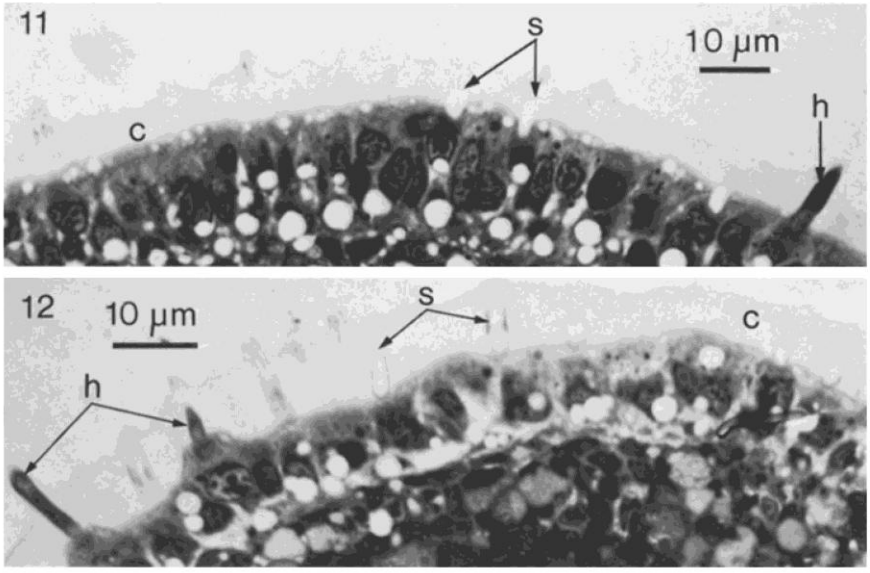

Fig. 11. Transverse section through the girdle of an 11 day-old juvenile. The spicules (*s*) and hairs (*h*) within the cuticle (*c*), scallop the cuticular surface. The spicules do not yet lie completely within the cuticle. ×1070

Fig. 12. Frontal section through the girdle of a 13 day-old juvenile. The spicules (*s*) lie completely wihin the cuticle (*c*). Two hairs (*h*) also lie within the cuticle. ×1170

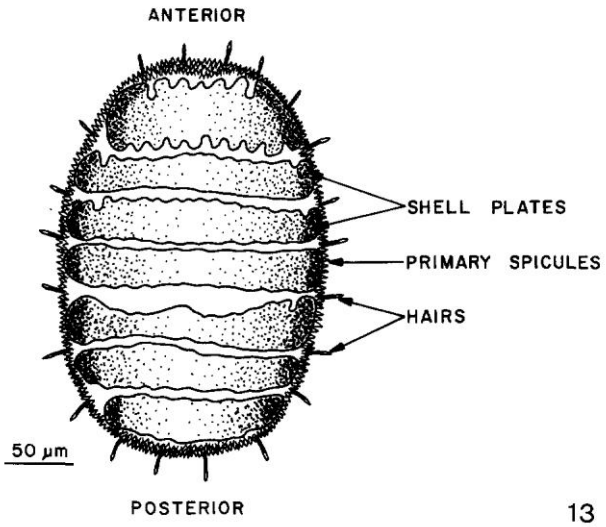

Fig. 13. Diagram of a young juvenile about two weeks old. The cuticle has been omitted for clarity. Hairs are produced around the entire margin of the girdle. This is the first row of hairs. Lateral hairs occur at the sutural margins, where two shells meet. A calcareous spikelet caps the tip of each hair. The eighth (posterior) shell plate will be secreted during the seventh week

Table 2. Metamorphic events occurring within 24 h of larval settlement in *Mopalia muscosa*

Events of Metamorphosis in Mopalia muscosa
Initiation of the loss of a functional prototroch
Loss of the apical tuft
Secretion of a cuticle over the mantle field
Initiation of the secretion of calcareous shell plates
Extrusion of the spicules into the cuticle
Decrease in length to about 80% of the larval length
Secretion of chitinous hairs
Incorporation of the lateral ciliated bands into the pallial grooves

Each chitinous hair shaft is produced by one trichogenous cell (Fig. 12) and has a small calcareous spikelet, about 2 μm wide and 4-8 μm long, at its tip (Fig. 7b). The apex of a trichogenous cell often bulges above the apices of the surrounding cells (Fig. 12). Hairs of young juveniles tend to be directly laterally or dorso-laterally and are easily eroded as the animal moves through the debris on the substratum.

D. Discussion

At least eight characteristic events that distinguish juveniles of *Mopalia muscosa* from larvae (Table 2) are manifested routinely within 24 h of settlement. These changes are interpreted as evidence of metamorphosis.

How widespread is metamorphosis among the chitons? Various researchers have studied the development of larvae and young juveniles of several chitons (Kowalevsky 1883; Heath 1899; Hammarsten and Runnstrom 1926; Grave 1932; Okuda 1947; Christiansen 1954; Matthews 1956; Barnes 1972; Watanabe and Cox 1975). These authors described the transformation of larvae into juveniles but did not always identify this transition as metamorphosis (Table 3). One major confounding factor is that the species were raised under different conditions, and often without any specific substratum, other than the bottom of the culture dish, for their juvenile existance. But do chitons need a specific substratum for settlement and the initiation of metamorphosis?

Like Watanabe and Cox (1975), I had first offered mussel shells covered with algal films to larval *M. muscosa*, but found that most animals did not metamorphose on these shells. Instead, red or brown algal mats on small stones promoted the development of juveniles. In my studies, *M. muscosa* settled, metamorphosed and grew for over a year on *Hildenbrandia* sp., a crustose, non-coralline, red alga that is a preferred substratum (Morse DE, personal communication; Leise, unpublished).

Tonicella lineata (see Barnes and Gonor 1973), *M. muscosa* (see Morse et al. 1979), and *Katharina tunicata* (see Rumrill and Cameron 1983) prefer to settle on specific algal substrata. *T. lineata* and *K. tunicata* metamorphose similarly to *M. muscosa* and do so only after settling (Barnes and Gonor 1973; Rumrill and Cameron 1983). Thus, at least three species of chitons metamorphose after settling onto specific substrata. Although other species may be less specific in their substratum requirements, an analysis of any species' development without considering its settlement needs may yield spurious results. For example, Okuda (1947) saw *Cryptochiton stelleri* settle just below the meniscus in the culture dishes. I have only observed animals there that are trapped by bacterial debris. *Lepidochitona cinerea's* lack of development past the trochophore

stage in sterile cultures (Matthews 1956) also indicates that this species needs an algal substratum to develop normally.

Table 3. Metamorphic changes in various species of chitons as reported (+) in the literature. These changes occur after the larva has settled. Changes not reported, (−)

Species	Loss of prototroch and apical tuft	Extrusion of spicules	Secretion of shell plates	Formation of pallial grooves	Growth of mantle field over head vesicle	Dorso-ventral flattening	Devel-opment of radular sac
Lepidopleuridae:							
Lepidopleurus asellus [Christiansen 1954]	+	+	−	−	−	−	−
Ischnochitonidae:							
Stenoplax heathiana [Heath 1899]	+	−	+	+	+	−	−
Lepidochitonidae:							
Tonicella lineata [Barnes 1972]	+	−	+	−	−	−	−
Lepidochitona cinerea [Haas et al. 1978]	+	−	+	+	−	−	−
Callistoplacidae:							
Middendorffia polii [Kowalevsky 1883]	+	+	+	−	−	−	−
Chaetopleuridae:							
Chaetopleura apiculata [Grave 1932]	+	−	−	−	−	+	−
Mopaliidae:							
Mopalia lignosa *Mopalia muscosa* [Watanabe and Cox 1975]	+	−	+	+	−	−	−
Katharina tunicata [Rumrill and Cameron 1983]	+	+	+	−	−	−	+
Acanthochitonidae:							
Acanthochiton discrepans [Hammarsten and Runnström 1926]	+	+	+	−	−	−	−
Cryptochiton stelleri [Okuda 1947]	+	−	−	−	−	+	−

If the available substrata are inappropriate, some molluscs can delay settling and may even continue to differentiate precociously during this delay (Pechenik 1980). Thus, chiton larvae that are cultured without an appropriate substratum may display " metamorphic" changes while still larvae; changes that would, under optimal conditions, only occur after settlement. *Cryptochiton stelleri* (see Okuda 1947) and *Chaetopleura apiculata* (see Grave 1932), were reported to secrete shell while still larvae. Both authors raised animals without a substratum for settlement. Their larvae may have delayed settlement while continuing to progress along their developmental programs.

With the exception of certain species specific changes (such as the development of the girdle hairs), the metamorphic changes I found in *M. muscosa* (Table 2) may be common to many chitons. The loss of a functional prototroch and apical tuft at metamorphosis occurs in many species (Table 3). These processes are not always instantaneous. In *Mopalia muscosa* loss of the prototroch begins at metamorphosis, but some metamorphosed individuals retain remnants of the prototroch for three to four days after settlement. The secretion of the cuticle and the extrusion of spicules into it had not been recognized as metamorphic changes until now. Larval *M. muscosa* are covered by a dense brush border of microvilli and do not produce a cuticle like that of the juveniles. Kowalevsky (1883) described a cuticle covering larval *Middendorffia polii*, but he may have mistaken the brush border for a cuticle, as it is difficult to resolve the microvilli, especially in wax-embedded material. Haas et al. (1978) observed no cuticle in larval *Lepido-chitona cinerea* but described one covering the dorsal surface of " bottom living larvae", that are probably metamorphosed juveniles. No distinction was made between extruded and unextruded spicules in the descriptions of development of *Mopalia muscosa* and *M. lignosa* (see Watanabe and Cox 1975), *Chaetopleura apiculata* (see Grave 1932), and

Cryptochiton stelleri (see Okuda 1947). Christiansen's (1954) observation that spicules become "more distinct " in *Lepidopleurus asellus* at metamorphosis, and Rumrill and Cameron's (1983) observation that spicules develop at metamorphosis in *K. tunicata* probably mean that spicules are also extruded at metamorphosis in these species. I ob-served extruded spicules only in metamorphosed juveniles, as did Kowalevsky (1883) in *M. polii.* Heath (1899) illustrates extruded spicules in *Stenoplax heathiana,* but does not discuss them. Unlike the situation in *M. mucosa,* spicules form after the first shell plates in *Acanthochiton discrepans* (see Hammarsten and Runnstrom 1926).

As in *Katharina tunicata* (see Rumrill and Cameron 1983), *Tonicella lineata* (see Barnes 1972) and *Middendorffia polii* (see Kowalevsky 1883), shell secretion in *Mopalia muscosa* does not begin until after settlement. It is one of the most obvious transformations of metamorphosis.

When *M. muscosa* metamorphoses, it shortens to about 80% of the larval length with a concurrent change to an oval shape. Grave (1932) saw a similar shortening and broadening of the body in metamorphosing *Chaetopleura apiculata.*

The sensory hairs of *Mopalia muscosa* develop at metamorphosis. Watanabe and Cox (1975) illustrated juvenile *M. muscosa* with large, unlabelled, marginal projections that are probably hairs, although they did not describe them. The hairs of young juvenile *Mopalia muscosa* are unlike those of the adults (Leise and Cloney 1982; Leise 1983). The morphogenesis of these hairs and of related sensory structures in the girdle will be described in the next paper of this series.

The coincidence of metamorphic changes in species from 7 of the 13 families (Bergenhayn 1955; Ferreira 1982) suggests the hypothesis that all chitons metamorphose in a similar way (Table 3). My study is limited to epidermal transformations; a study of the internal organs may reveal additional changes. As other species are examined, differences in their metamorphic changes may become apparent, but to achieve optimal results such studies should simulate the environmental conditions needed by these animals as closely as possible.

The dramatic metamorphosis of an organism from a pelagic larva to a benthic juvenile is considered to be an ancestral molluscan feature (Morton 1967). The loss of, or a reduction in the metamorphic changes usually occurs in more highly evolved molluscan taxa. The chitons are primitive molluscs — retaining many of the presumed " ancestral" phyletic characteristics (Morton 1967). The retention of a series of distinctive metamorphic changes through-out the class would yield further evidence for their "primitive" state.

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